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25. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action:

catalyzing the following reaction:

creatine + $H_2O \rightarrow \text{sarcosine} + \text{urea}$

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:

3.5-10.0 mM.

33. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action:

catalyzing the following reaction:

creatine + H₂O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

35. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

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Action:catalyzing the following reaction:

creatine + $H_2O \rightarrow sarcosine + urea$

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a

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peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

36. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action:

catalyzing the following reaction:

creatine + $H_2O \rightarrow \text{sarcosine} + \text{urea}$

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

37. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid-sequence of SEQ-ID-NO:2-or-(b)-anucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + $H_2O \rightarrow sarcosine + urea$

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

